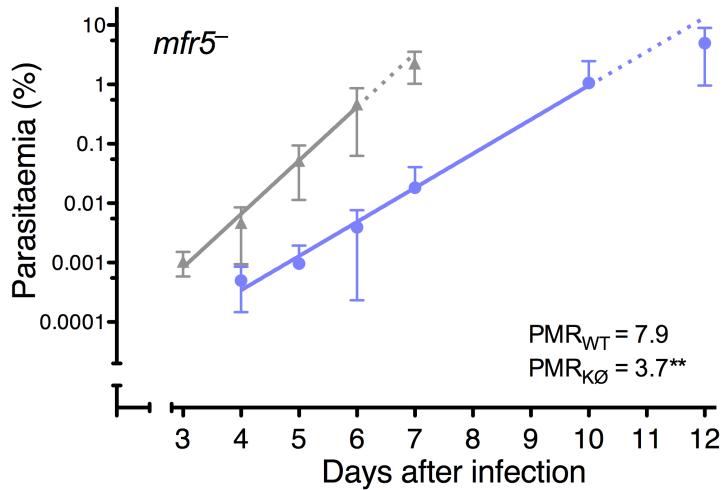


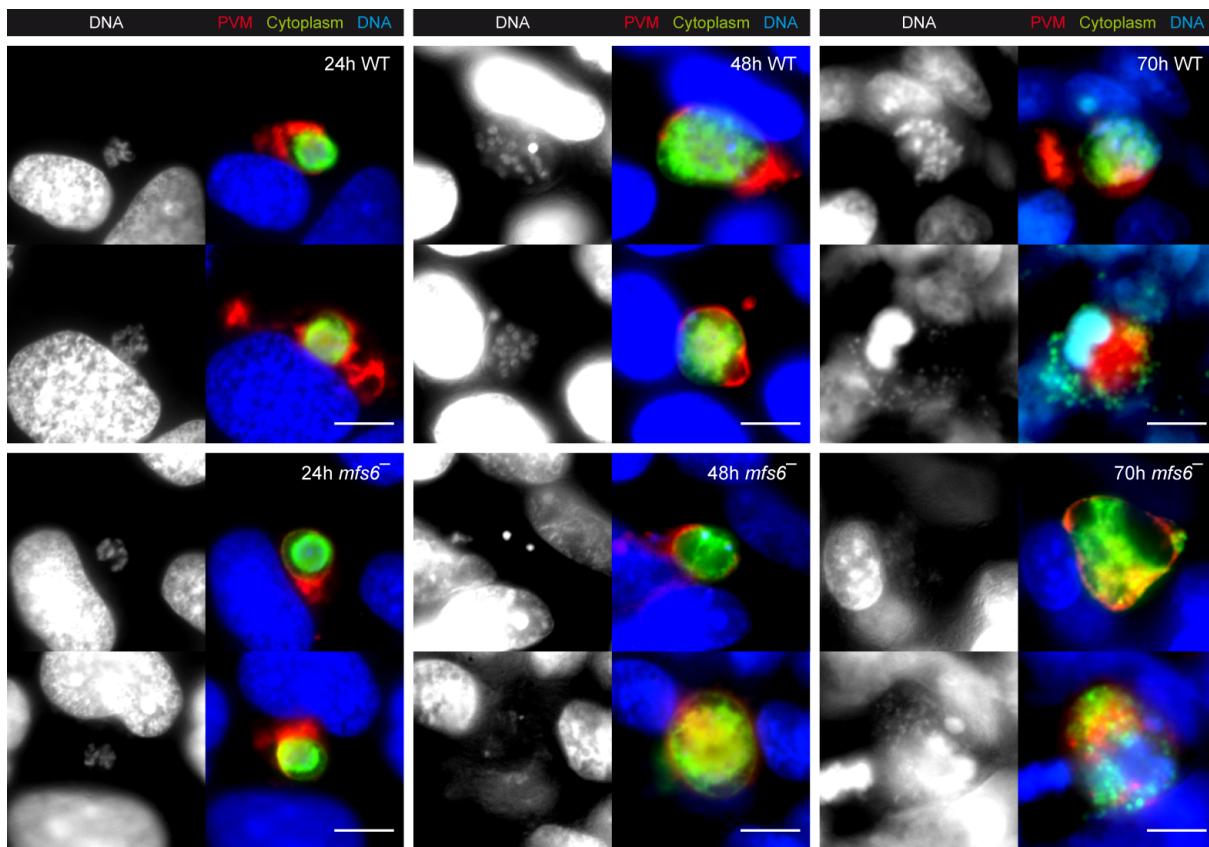
Supplementary Figure 1 | Comparison of membrane transport protein (MTP) type distribution in different organisms. Shown is the percentage of channels, pumps, and porters of the total MTPs in different organisms. The percentages on top of the bars indicate the proportion of all open reading frames in the respective genomes encoding MTPs (data from [http://www.membranetransport.org]¹).



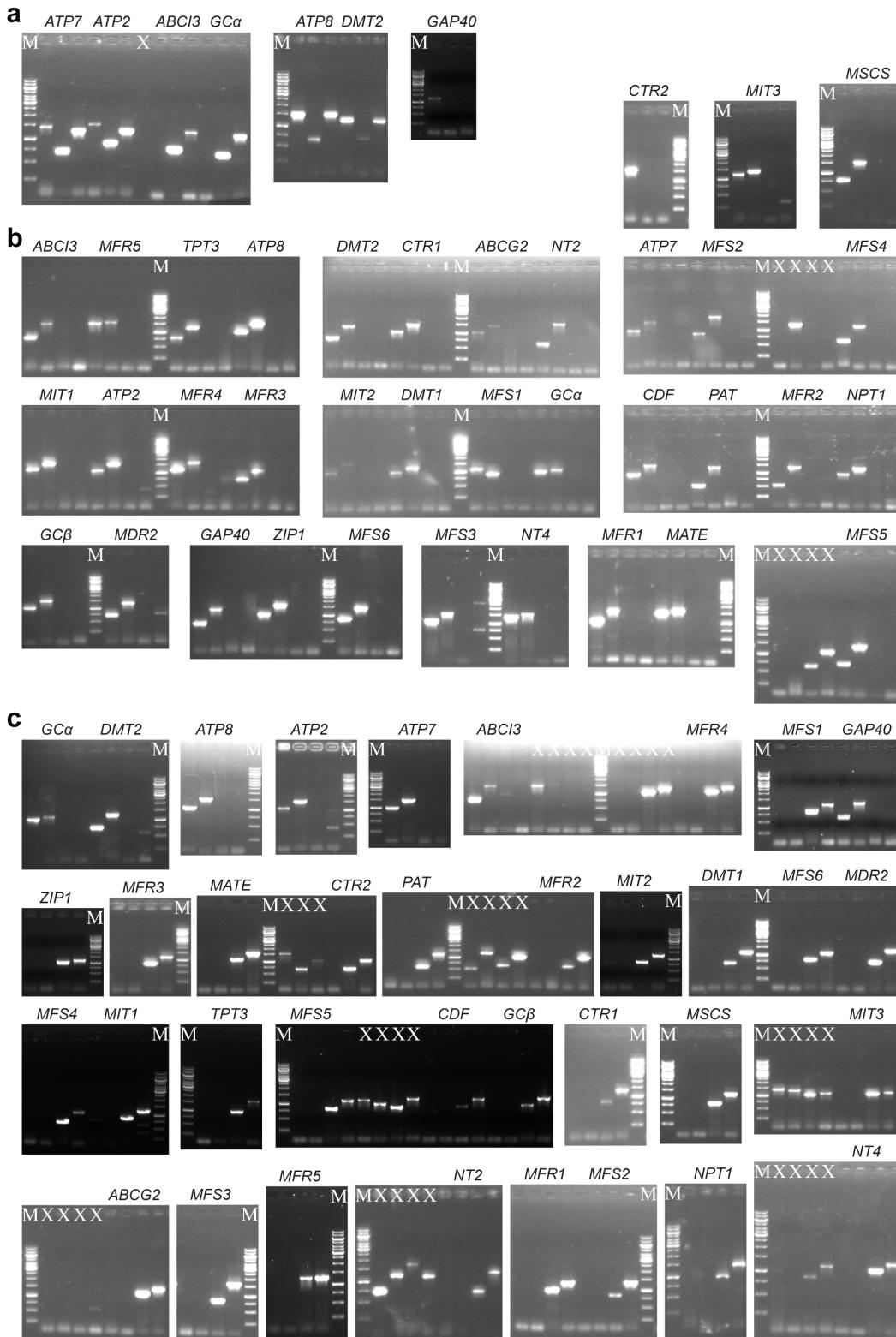
Supplementary Figure 2 | The glideosome associated protein 40 (GAP40) locus is inaccessible to genetic manipulation. Diagnostic PCR revealed absence of the desired gene replacement or endogenous tagging and presence of WT-specific PCR products (centre and right, red boxes) similar to those of the recipient parasites (left, black boxes; see Supplementary Fig. 5 for full gel pictures). Transfactions were performed three times with the pMTP-KO vectors (red cross) and in the case of the gene deletion attempt once more with the respective *PlasmoGEM* vector (pink cross). Although inaccessibility of the *GAP40* locus to genetic manipulation cannot be formally excluded, it is likely essential in asexual blood stage parasites while endogenous tagging of the membrane-associated protein with the mCherry-3xMyc tag interferes with its function. In *Toxoplasma gondii*, GAP40 is associated with the glideosome².



Supplementary Figure 3 | Individual blood infection of *mfr5*⁻ parasites. Blood infection of the slowest growing parasite line, *mfr5*⁻ (violet) compared with WT (grey) when grown in individual mice. Shown are mean parasitaemias \pm S.D. from three experiments monitored by flow cytometry. Data from the exponential growth phase, *i.e.* with parasitemia <1%, fitted a linear regression well ($r^2 \geq 0.99$) and allowed the calculation of the parasite multiplication rate (PMR) from the slopes. **, p<0.01; (two-tailed F-test).



Supplementary Figure 4 | Aberrant liver-stage maturation of *mfs6*⁻ parasites. Shown are two representative immunofluorescent micrographs each of cultured hepatoma cells at 24, 48, and 70 h after infection with WT or *mfs6*⁻ salivary gland-associated sporozoites. (UIS4, parasite vacuole membrane, red; HSP70, cytoplasm, green; Hoechst, DNA, blue). Bars, 10 μ m.



Supplementary Figure 5 | Full gel pictures. Diagnostic PCR of (a) parental populations with endogenously tagged genes, (b) WT parasites with the intact gene loci, and (c) parental and isogenic populations for essential and targetable genes, respectively, after transfection. M, lanes with 1kb DNA marker; X, lanes irrelevant to this study. The primers used from left to right are for 5' and 3' WT, 5' and 3' integration (note that for tagging genes and *CTR2* a single overarching WT PCR was performed).

Supplementary Table 1 | *Plasmodium berghei* genes encoding membrane transport proteins targeted in this study (Fig 1a).

Gene	Pb ID	Pf ID	Predicted product	Ref
Channels/Pores – 1.A.23 The Small Conductance Mechanosensitive Ion Channel (MscS) Family				
MSCS	PBANKA_093900	PF3D7_1107900	mechanosensitive ion channel protein	
Channels/Pores – 1.A.35 The CorA Metal Ion Transporter (MIT) Family				
MIT1	PBANKA_092790	PF3D7_1120300	metal ion channel - Mg ²⁺ , Co ²⁺ and Ni ²⁺	3
MIT2	PBANKA_140270	PF3D7_1304200	CorA-like Mg ²⁺ transporter protein, putative	
MIT3	PBANKA_101700	PF3D7_1427600	CorA-like Mg ²⁺ transporter protein, putative	
Channels/Pores – 1.A.56 The Copper Transporter (Ctr) Family				
CTR1	PBANKA_130290	PF3D7_1439000	copper transporter	4
CTR2	PBANKA_102150	PF3D7_1421900	Ctr copper transporter domain containing protein, putative	
Primary Active Transporters/Pumps – 3.A.1: The ATP-binding Cassette (ABC) Superfamily				
ABCG2	PBANKA_101810	PF3D7_1426500	ATP-binding cassette sub-family G member 2 (ABCG2)	5
ABC1/3	PBANKA_121880	PF3D7_0319700	ABC transporter, putative	
MDR2	PBANKA_131170	PF3D7_1447900	multidrug resistance protein 2 (heavy metal transport family)	6
Primary Active Transporters/Pumps – 3.A.3 The P-type ATPase (P-ATPase) Superfamily				
ATP2	PBANKA_143480	PF3D7_1219600	aminophospholipid-transporting P-ATPase (ATPase2)	6
ATP7	PBANKA_080630	PF3D7_0319000	P-type ATPase, putative (ATPase7)	
ATP8	PBANKA_143830	PF3D7_1223400	phospholipid-transporting ATPase, putative (ATPase8)	
GCa	PBANKA_091030	PF3D7_1138400	aminophospholipid-transporting P-ATPase & guanylyl cyclase domains	7,8
GC β	PBANKA_113670	PF3D7_1360500	aminophospholipid-transporting P-ATPase & guanylyl cyclase domains	9-11
No Pb orthologue		PF3D7_1468600	aminophospholipid transporter, putative	
Electrochemical Potential-driven Transporters – 2.A.1 The Major Facilitator Superfamily (MFS)				
MFS1	PBANKA_123130	PF3D7_0516500	metabolite/drug transporter, putative	
MFS2	PBANKA_081700	PF3D7_0916000	sugar transporter, putative	10,11
MFS3	PBANKA_082040	PF3D7_0919500	sugar transporter, putative	
MFS4	PBANKA_060240	PF3D7_1203400	transporter, putative	
MFS5	PBANKA_101640	PF3D7_1428200	metabolite/drug transporter, putative	
MFS6	PBANKA_130470	PF3D7_1440800	major facilitator superfamily, putative	
PAT	PBANKA_030390	PF3D7_0206200	pantothenate transporter	12,13
Electrochemical Potential-driven Transporters – The Major Facilitator Superfamily Related Transporters (MFR)				
MFR1	PBANKA_011250	PF3D7_0614300	organic anion transporter	
MFR2	PBANKA_020840	PF3D7_0104700	transporter, putative	
MFR3	PBANKA_041050	PF3D7_0312500	transporter, putative	
MFR4	PBANKA_081570	PF3D7_0914700	transporter, putative	
MFR5	PBANKA_091830	PF3D7_1129900	transporter, putative	
NPT1	PBANKA_020830	PF3D7_0104800	novel putative transporter 1	14
No Pb orthologue		PF3D7_0529200	sugar transporter, putative	
Electrochemical Potential-driven Transporters – 2.A.4 The Cation Diffusion Facilitator (CDF) Family				
CDF	PBANKA_142220	PF3D7_0715900	zinc transporter, putative	
Electrochemical Potential-driven Transporters – 2.A.5 The Zinc (Zn²⁺)-Iron (Fe²⁺) Permease (ZIP) Family				
ZIP1	PBANKA_010770	PF3D7_0609100	Zn ²⁺ or Fe ²⁺ permease	
Electrochemical Potential-driven Transporters – 2.A.7 The Drug/Metabolite Transporter (DMT) Superfamily				
DMT1	PBANKA_142210	PF3D7_0715800	drug/metabolite exporter, drug/metabolite transporter	
DMT2	PBANKA_061460	PF3D7_0716900	drug metabolite transporter, putative	
GAP40	PBANKA_111530	PF3D7_0515700	glideosome-associated protein 40, putative	
TPT3	PBANKA_143400	PF3D7_1218400	ribose or hexose phosphate/phosphate translocator, putative	3
Electrochemical Potential-driven Transporters – 2.A.57 The Equilibrative Nucleoside Transporter (ENT) Family				
NT2	PBANKA_070620	PF3D7_0824400	nucleoside transporter 2	15
No Pb orthologue		PF3D7_1469400	nucleoside transporter 3, putative (NT3)	
NT4	PBANKA_020990	PF3D7_0103200	nucleoside transporter 4	16
Electrochemical Potential-driven Transporters – 2.A.66.1 The Multi Antimicrobial Extrusion (MATE) Family				
MATE	PBANKA_030970	PF3D7_0212800	multidrug efflux pump, putative	

Supplementary Table 2 | Details of the phenotyping screen (Fig. 1c and 3).

	Parasitemia (%)		Exflagellation (events/ μ l blood)		Sporozoites/female mosquito		Transmission (days after infection)	
<i>MSCS</i>	10.2	6.1	12,300	nd	900	1,100	3	3
<i>MIT1</i>	9.4	6.1	16,000	nd	0	1,500	4	4
<i>MIT2</i>	15.3	19.0	18,600	20,000	0	0	-	-
<i>MIT3</i>	9.5	6.8	11,300	nd	3,500	7,500	4	4
<i>CTR1</i>	10.7	15.7	11,600	nd	0	700	-	-
<i>CTR2</i>	11.6	13.9	2,600	11,300	67,100	4,300	-	-
<i>ABCG2</i>	7.9	7.7	21,000	25,300	40,000	7,100	3	4
<i>MDR2</i>	11.0	7.1	15,300	nd	900	7,500	6	-
<i>GCβ</i>	6.2	5.7	8,600	nd	0	0	-	-
<i>MFS1</i>	9.1	9.5	11,300	11,400	0	500	-	-
<i>MFS2</i>	6.7	6.9	16,600	nd	0	0	-	-
<i>MFS3</i>	9.5	12.3	7,100	nd	500	1,600	4	5
<i>MFS4</i>	10.6	10.4	16,700	19,700	10,500	16,600	3	3
<i>MFS5</i>	7.9	6.8	6,300	nd	2,100	6,500	3	3
<i>MFS6</i>	4.1	4.5	3,700	4,700	12,100	20,000	-	-
<i>PAT</i>	6.3	7.4	2,800	1,500	0	0	-	-
<i>MFR1</i>	12.1	14.3	11,200	11,300	27,500	21,300	3	3
<i>MFR2</i>	4.4	3.5	5,000	nd	15,000	6,800	5	-
<i>MFR3</i>	9.5	9.0	6,000	nd	400	800	3	5
<i>MFR4</i>	12.8	9.5	15,300	nd	0	0	-	-
<i>MFR5</i>	0.9	1.3	0 ^a	100 ^a	0	0	-	-
<i>NPT1</i>	4.6	5.8	100	1,000	0	0	-	-
<i>CDF</i>	7.7	7.0	1,300	400	1,100	0	4	-
<i>ZIP1</i>	3.0	2.3	0	0	0	0	-	-
<i>DMT1</i>	6.9	3.5	21,800	nd	4,100	1,000	4	-
<i>TPT3</i>	6.3	9.2	15,100	nd	5,800	12,900	3	4
<i>NT2</i>	7.2	10.5	6,600	10,500	7,000	4,100	4	4
<i>NT4</i>	10.3	9.4	14,700	nd	0	0	-	-
<i>MATE</i>	11.8	12.8	27,300	nd	500	1,000	4	4
WT median	7.6		14,400		24,500		4	
<i>n</i>	22		16		14		14	
WT 10% percentile	4.6		5,900		2,500		- (n=1) ^b	
WT 25% percentile	6.1		6,800		6,900		5 (n=1) ^b	
WT 75% percentile	9.3		18,300		40,400		4 (n=7) ^b	
WT 90% percentile	10.8		25,300		50,100		3 (n=5) ^b	

^a Due to slow asexual blood stage multiplication rate, the screen was continued by injecting 6×10^7 *mfr5*⁻ infected erythrocytes instead of 1×10^7 , the inoculum for all other recombinant parasite lines. Parasitemias of the two mice, from which exflagellation was assessed, was 5.6 and 7.5%, respectively.

^b Since mice remaining blood-film negative following infectious mosquito bites preclude the calculations of correct values for the percentile cut-offs, the counts of the prepatent periods of WT infections are indicated.

Supplementary Table 3 | Primer sequences and PCR product sizes.

Primer Name	Primer Sequence (restriction sites underlined)	WT ^a	INT ^b	Use ^c	Target	Ref
Primers of general use						
GFPrev	TGTGCCCATTAACATCACCATC			GT	GFP	17
mCherryRev	CCCTCCATTGAAACCTTGAAG			GT	mCherry	18
5'HSP70rev	CAATTGTTGACATAAAATAGGCAG			GT	5'PbHSP70	18
5'DHFRrev	ATGAAATACCGCTCCATTTC			GT	5'PbDHFR-TS	18
pBAT-SQF	TATTGCTAACTATATTCAAGGG			SQ	PbSIL6L	
pBAT-SQR	TTATTGCTGCTGAATTATAGTGC			SQ	PbSIL6R	
T7	TAATACGACTCACTATAGGG			GT	pBSKS	
MSCS – PBANKA_093900 – PF3D7_1107900 – mechanosensitive ion channel protein						
5'MSCS-F-SaclI	TTTCCGGGCCATTATGTGCGTCTGAATCC		0.5	TV	5'PbMSCS	
5'MSCS-R-Hpal	TTTCAGCTTAAATTAAAGCGCATAATCGTTTATTAC			TV	5'PbMSCS	
3'MSCS-F-AvrII	AAT <u>CCTAGG</u> TCTAAAATAAAGATTTCAGTGG		0.5	TV	3'PbMSCS	
3'MSCS-R-KpnI	ATAGGT <u>ACCG</u> GTCAAACAATGTGGTAGTCACC			TV	3'PbMSCS	
5'MSCS-F	AAGAGAGAAAGCTGGATAATGC		0.8	GT	5'PbMSCS	
5'MSCS-R	TGATTGTTGATACTTTGCTTCG			GT	5'PbMSCS	
3'MSCS-F	TGGACAAAATGAAATGGGTGG		1.6	GT	3'PbMSCS	
3'MSCS-R	TGGCTAAAAGCTGTGAAATGG		1.4	GT	3'PbMSCS	
MIT1 – PBANKA_092790 – PF3D7_1120300 – metal ion channel - Mg²⁺, Co²⁺ and Ni²⁺						
5'MIT1-F-SaclI	TTTCCGGCGATATAGATTACACAAACTCATACCG		0.6	TV	5'PbMIT1	
5'MIT1-R-Hpal	TTTCAGCTTAAATTAAATTATATATAACACAAATAATTTATAGAAAAATTATCG			TV	5'PbMIT1	
3'MIT1-F-AvrII	AAT <u>CCTAGG</u> GAGCATGTGAATATCACCAATATGC		0.5	TV	3'PbMIT1	
3'MIT1-R-KpnI	ATAGGT <u>ACCC</u> ACTGTATTCAAGACAGAAATTGC			TV	3'PbMIT1	
5'MIT1-F	CTCACCTAACAAATACTTGG		0.9	GT	5'PbMIT1	
5'MIT1-R	CAAATAGCCAAGTTTCAATTATATCG			GT	5'PbMIT1	
3'MIT1-F	AGGAATTGTACAGATAATGGAAAGG		1.6	GT	3'PbMIT1	
3'MIT1-R	GATATATAAAATTATCATTACAATTGATGG		1.4	GT	3'PbMIT1	
MIT2 – PBANKA_140270 – PF3D7_1304200 – CorA-like Mg²⁺ transporter protein, putative						
5'MIT2-F-SaclI	TTTCCGGGATTAAATCGTTGGTTATTACATATGC		0.6	TV	5'PbMIT2	
5'MIT2-R-Hpal	TTTGTTAA <u>CTTAA</u> TTAAAAATTATCAAAGGGATGACGAG			TV	5'PbMIT2	
3'MIT2-F-AvrII	AAT <u>CCTAGG</u> CGCTAACGTACGAATAAAATGG		0.5	TV	3'PbMIT2	
3'MIT2-R-KpnI	ATAGGT <u>ACCC</u> AAATTAAATAGAAAATGAGAAACACG			TV	3'PbMIT2	
5'MIT2-F	AATATTGAAATTCTCCCAACTGC		1.1	GT	5'PbMIT2	
5'MIT2-R	TTATTCTAAATGTTGCTCATTC			GT	5'PbMIT2	
3'MIT2-F	TATCAAATTACATAACCTAAAGAGGCC		1.5	GT	3'PbMIT2	
3'MIT2-R	CACATCTCATTTCACAGATGC		1.4	GT	3'PbMIT2	
MIT3 – PBANKA_101700 – PF3D7_1427600 – CorA-like Mg²⁺ transporter protein, putative						
5'MIT3-F-SaclI	TTTCCGGGAATTATGTTTTATACACGGAAATTGC			TV	5'PbMIT3	
5'MIT3-R-Hpal	TTTGTTAA <u>CTTAA</u> TTAAATAATAGATCAATATATAATATGCGAATGC		0.6	TV	5'PbMIT3	
3'MIT3-F-AvrII	AAT <u>CCTAGG</u> GAATATTTATGCAATATCCTTCG			TV	3'PbMIT3	
3'MIT3-R-KpnI	ATAGGT <u>ACCC</u> AAATTGTAAAATGAACATTGATGC		0.5	TV	3'PbMIT3	
5'MIT3-F	AAACATTATGGAAGATACTGTGC		1.3	GT	5'PbMIT3	
5'MIT3-R	TGCAAAATTAGTAAATTATGGG			GT	5'PbMIT3	
3'MIT3-F	CCCACATTACAAATAACCCCTATACC		1.4	GT	3'PbMIT3	
3'MIT3-R	GCTGTAGATTGTGTTTATTTTCG		1.3	GT	3'PbMIT3	
CTR1 – PBANKA_130290 – PF3D7_1439000 – copper transporter						
5'CTR1-F-SaclI	TTTCCGGGAAATAATCCATTAAAGATAATAAAATCGC			TV	5'PbCTR1	
5'CTR1-R-Hpal	TTTGTTAA <u>CTTAA</u> TTAAAGGTTAGTTATTGTTGTTATTGATAAG		0.5	TV	5'PbCTR1	
3'CTR1-F-AvrII	AAT <u>CCTAGG</u> ACAAGATATTATTCTATATGTTGACTCC			TV	3'PbCTR1	
3'CTR1-R-KpnI	ATAGGT <u>ACCC</u> ATTATTCTCTATTAAAGATATGGTTCC		0.5	TV	3'PbCTR1	
5'CTR1-F	TGTTGTTGGCGTAAAAATGTGC			GT	5'PbCTR1	
5'CTR1-R	GAATTTGTTTTGCCTTCACTCC		1.0	GT	5'PbCTR1	
3'CTR1-F	GCCTATGTCATTCAGAATACTACC			GT	3'PbCTR1	
3'CTR1-R	TATACGAAGCGACAATAAAATGC		1.4	GT	3'PbCTR1	
1.5						
CTR2 – PBANKA_102150 – PF3D7_1421900 – Ctr copper transporter domain containing protein, putative						
5'CTR2-F-SaclI	TTTCCGGGTTCTGTTAAATCCATTAAAGATAATAAAATATGC		0.5	TV	5'PbCTR2	
5'CTR2-R-Hpal	TTTGTTAA <u>CTTAA</u> TTAAATTAGAAATTGGTATATCTTAAATTATCATTG			TV	5'PbCTR2	
3'CTR2-F-AvrII	AAT <u>CCTAGG</u> TAACAACTGTACTCAACATATTAAAGC		0.5	TV	3'PbCTR2	
3'CTR2-R-KpnI	ATAGGT <u>ACCC</u> ATATACATAATATAATGGACAGATTAAACC			TV	3'PbCTR2	
5'CTR2-F	TTAACAAATGTTGCACAAATAGTGG		1.6 ^d	GT	5'PbCTR2	
3'CTR2-R	TCAAATAAGATGGTAGCTAAATAGG		1.3	GT	3'PbCTR2	

Supplementary Table 3 | Primer sequences and PCR product sizes (continued...)

Primer Name	Primer Sequence (restriction sites underlined)	WT ^a	INT ^b	Use ^c	Target	Ref
ABCG2 – PBANKA_101810 – PF3D7_1426500 – ATP-binding cassette sub-family G member 2 (ABCG2)						
5'ABCG2-F-SaclI	TTT <u>CCGCGGT</u> GAACATGCGGGAAAAAGTATGC	0.5		TV	5'PbABCG2	
5'ABCG2-R-Hpal	TTTGTTAAC <u>TTAA</u> ACGTGGAAAAATATGGAAATGAG			TV	5'PbABCG2	
3'ABCG2-F-AvrlI	AAT <u>CCCTAGG</u> ATATT <u>ATA</u> TTAAAGTGTATTGCCATATCC	0.5		TV	3'PbABCG2	
3'ABCG2-R-KpnI	AT <u>AGGTACCAAA</u> ATT <u>ACG</u> GCTAGCTAAATTGG			TV	3'PbABCG2	
5'ABCG2-F	GAAGTGAACGAAAATGAATATAAGC	1.1	1.2	GT	5'PbABCG2	
5'ABCG2-R	TGATGTCAA <u>ATC</u> TTT <u>AT</u> TTTATGC			GT	5'PbABCG2	
3'ABCG2-F	ACATCGGGTTTAGATTCAATGG	1.5		GT	3'PbABCG2	
3'ABCG2-R	TTCTATTTACGTGGTTGACTTAC		1.3	GT	3'PbABCG2	
ABC13 – PBANKA_121880 – PF3D7_0319700 – ABC transporter, putative						
5'ABC13-F-SaclI	TTT <u>CCGCGG</u> AAAATA <u>ACA</u> ATAGAAAAGATAAAGAAC	0.5		TV	5'PbABC13	
5'ABC13-R-Hpal	TTTGTTAAC <u>TTAA</u> TAAGAGTTATTTAAAAATATAATTAA <u>TTT</u> ATTAGCAAAG			TV	5'PbABC13	
CT-ABC13-F-SaclI	AAT <u>CCGCGG</u> TTAA <u>ATTG</u> ATTGATAAT <u>ACT</u> TTCTTATGTTC	0.5		TV	CT-PbABC13	
CT-ABC13-R-Hpal	AAAGTTAAC <u>CCG</u> CTT <u>CAA</u> ATCAATTAGGAAAATATC			TV	CT-PbABC13	
3'ABC13-F-AvrlI	AAT <u>CCCTAGG</u> TT <u>CC</u> AAA <u>ACG</u> TGGAAAAATAAAC	0.5		TV	3'PbABC13	
3'ABC13-R-KpnI	AT <u>AGGTACCC</u> TT <u>ATG</u> TACAGTCATTACAAACAAGG			TV	3'PbABC13	
5'ABC13-F	TGAAA <u>AT</u> CAGTC <u>TT</u> TATAAGC	0.8	0.9	GT	5'PbABC13	
5'ABC13-R	CT <u>CTT</u> TT <u>TTA</u> AG <u>CC</u> AT <u>ATG</u> TTACG			GT	5'PbABC13	
CT-ABC13-F	as 3'ABC13-F	1.5	0.8	GT	CT-PbABC13	
3'ABC13-F	GATTTGAA <u>ATT</u> CCTGAAA <u>AGT</u> ATGC			GT	3'PbABC13	
3'ABC13-R	TTGTT <u>AA</u> GAATA <u>TTT</u> GAGGAGC	1.5	1.5	GT	3'PbABC13	
MDR2 – PBANKA_131170 – PF3D7_1447900 – multidrug resistance protein 2 (heavy metal transport family) (MDR2)						
5'MDR2-F-SaclI	TTT <u>CCGCGG</u> AAAATAGGATTAAAAATATTACTTA <u>ACTG</u> TATATATAG	n/a		TV	5'PbMDR2	
5'MDR2-R-Hpal	TTTGTTAAC <u>TTAA</u> AT <u>GT</u> AT <u>GC</u> CTTTGTAAA <u>AAA</u> AACC			TV	5'PbMDR2	
3'MDR2-F-AvrlI	AAT <u>CCCTAGG</u> TT <u>GG</u> AA <u>GT</u> TA <u>ATC</u> CAAA <u>ACTA</u> ATCG	0.5		TV	3'PbMDR2	
3'MDR2-R-KpnI	AT <u>AGGTACCA</u> AT <u>ATG</u> CTT <u>ATT</u> AT <u>ATC</u> GT <u>ATG</u> GG			TV	3'PbMDR2	
5'MDR2-F	GT <u>GC</u> AT <u>AT</u> TC <u>GG</u> AAA <u>ACG</u>		n/a	GT	5'PbMDR2	
5'MDR2-R	T <u>CCG</u> AAA <u>ATG</u> GT <u>CT</u> T <u>AA</u> GG			GT	5'PbMDR2	
3'MDR2-F	T <u>AT</u> AT <u>CG</u> TT <u>TT</u> AT <u>ATG</u> GT <u>CT</u> GA <u>AGG</u>	1.4		GT	3'PbMDR2	
3'MDR2-R	CA <u>AAA</u> AT <u>CTG</u> GA <u>AT</u> TT <u>AT</u> AC <u>AT</u> GC		1.5	GT	3'PbMDR2	
ATP2 – PBANKA_143480 – PF3D7_1219600 – P-type ATPase, putative (ATPase2)						
5'ATP2-F-SaclI	TTT <u>CCGCGG</u> CC <u>GG</u> GT <u>ACT</u> AA <u>TAAC</u> AT <u>CC</u>	0.6		TV	5'PbATP2	
5'ATP2-R-Hpal	TTTGTTAAC <u>TTAA</u> AA <u>AGC</u> AG <u>TG</u> AT <u>AT</u> AC <u>AC</u> CTG			TV	5'PbATP2	
CT-ATP2-F-SaclI	AAT <u>CCGCGG</u> CC <u>AG</u> GT <u>CC</u> TT <u>CG</u> TT <u>GG</u>	0.5		TV	CT-PbATP2	
CT-ATP2-R-Hpal	AA <u>AGT</u> TA <u>ACT</u> AA <u>GT</u> TT <u>ATC</u> GT <u>TT</u> CT <u>TA</u> GT <u>AA</u> ATG			TV	CT-PbATP2	
3'ATP2-F-AvrlI	AAT <u>CCCTAGG</u> TT <u>TC</u> TT <u>AGC</u> ATT <u>GG</u> CT <u>TA</u> AC <u>TT</u> CG	0.5		TV	3'PbATP2	
3'ATP2-R-KpnI	AT <u>AGGTACCG</u> GT <u>AT</u> GG <u>CT</u> TA <u>AC</u> TT <u>CG</u>			TV	3'PbATP2	
5'ATP2-F	T <u>GC</u> AT <u>TT</u> AA <u>AC</u> AT <u>TC</u> CA <u>TT</u> AT <u>AG</u> TC	1.2	1.1	GT	5'PbATP2	
5'ATP2-R	GA <u>AGT</u> AA <u>TC</u> GT <u>AA</u> TT <u>GT</u> T <u>ATG</u> GC			GT	5'PbATP2	
CT-ATP2-F	as 3'ATP2-F	1.6	0.9	GT	CT-PbATP2	
3'ATP2-F	AT <u>GGG</u> TT <u>AA</u> AT <u>AG</u> CT <u>TT</u> TC <u>AC</u> G			GT	3'PbATP2	
3'ATP2-R	AT <u>TTT</u> CC <u>CT</u> TT <u>AT</u> AT <u>GC</u> ACT <u>TC</u> G	1.6	1.4	GT	3'PbATP2	
ATP7 – PBANKA_080630– PF3D7_0319000 – aminophospholipid-transporting P-ATPase (ATPase7)						
5'ATP7-F-SaclI	TTT <u>CCGCGG</u> C <u>AT</u> TA <u>GT</u> AA <u>TT</u> CT <u>AA</u> TT <u>GC</u> TT <u>GC</u>	0.4		TV	5'PbATP7	
5'ATP7-R-Hpal	TTTGTTAAC <u>TTAA</u> AT <u>AT</u> CT <u>TT</u> GC <u>AC</u> AC <u>AA</u> AT <u>AC</u> CG <u>CG</u>			TV	5'PbATP7	
CT-ATP7-F-SaclI	AAT <u>CCGCGG</u> AC <u>CA</u> AT <u>GT</u> TT <u>AT</u> GC <u>CG</u> TT <u>AT</u> GC	0.4		TV	CT-PbATP7	
CT-ATP7-R-Hpal	AA <u>AGT</u> TA <u>CT</u> GT <u>TT</u> CT <u>TT</u> CA <u>AC</u> AT <u>AA</u> GA <u>AA</u> AT <u>GA</u> AT <u>TC</u>			TV	CT-PbATP7	
3'ATP7-F-AvrlI	AAT <u>CC</u> TA <u>GGG</u> CAT <u>AC</u> CG <u>CT</u> GT <u>AG</u> AAA <u>AT</u> TC	0.5		TV	3'PbATP7	
3'ATP7-R-KpnI	AT <u>AGGTAC</u> CT <u>AC</u> AT <u>AT</u> TT <u>GG</u> CC <u>AT</u> CC <u>AT</u> CC			TV	3'PbATP7	
5'ATP7-F	TT <u>AT</u> AT <u>CG</u> GT <u>AG</u> ACT <u>AA</u> AC <u>GG</u>	1.0	1.1	GT	5'PbATP7	
5'ATP7-R	AT <u>AA</u> AT <u>CC</u> GG <u>TT</u> CT <u>CA</u> TT <u>AT</u> TT <u>CC</u>			GT	5'PbATP7	
CT-ATP7-F	as 3'ATP7-F	1.5	0.7	GT	CT-PbATP7	
3'ATP7-F	AC <u>CA</u> TT <u>CT</u> AT <u>AT</u> TC <u>AC</u> AT <u>GA</u> TC <u>G</u>			GT	3'PbATP7	
3'ATP7-R	GT <u>AT</u> AT <u>CG</u> TT <u>TC</u> AT <u>TT</u> AC <u>AT</u> TC <u>G</u>	1.5	1.5	GT	3'PbATP7	

Supplementary Table 3 | Primer sequences and PCR product sizes (continued...)

Primer Name	Primer Sequence (restriction sites underlined)	WT ^a	INT ^b	Use ^c	Target	Ref	
ATP8 – PBANKA_143830 – PF3D7_1223400 – phospholipid-transporting ATPase, putative							
5'ATP8-F-SaclI	TTTCCGCGGGAAATAAATGAGTAAGTAGAATATCTCC	0.6		TV	5'PbATP8		
5'ATP8-R-Hpal	TTTGTTAACCTAATTAAATTTCATCAAATATTGCAATCCGTTC			TV	5'PbATP8		
CT-ATP8-F-SaclI	AATCCGCGGTAGGGTACTGTTATGCTAGG	0.5		TV	CT-PbATP8		
CT-ATP8-R-EcoRI	AAA <u>GAA</u> TTCAGCTAGCTAAAAAATTGTGATGG			TV	CT-PbATP8		
3'ATP8-F-AvrlI	AAT <u>CCT</u> AGGTACACTTTGTGTGAAATTATGCG	0.5		TV	3'PbATP8		
3'ATP8-R-KpnI	ATAGGTACCGGAATACATACCATCATTCTGTGAA			TV	3'PbATP8		
5'ATP8-F	ATGCTAGTAATTTCCTTTATTATGC		0.9	GT	5'PbATP8		
5'ATP8-R	CAAAGGAGCAACAAATGTAACACATACCC	1.0		GT	5'PbATP8		
CT-ATP8-F	as 3'ATP8-F		1.5	0.7	GT	CT-PbATP8	
3'ATP8-F	CAACATACCAAGAAATGCAATGC			GT	3'PbATP8		
3'ATP8-R	GTAATAAGAGTTGGAAAAGAAAACC		1.5	1.7	GT	3'PbATP8	
GCα – PBANKA_091030 – PF3D7_1138400 – guanylyl cyclase (GCalpha)							
5'GCα-F-SaclI	TTTCCGCGGTGTCACAAATATCACACACACC	0.5		TV	5'PbGCα		
5'GCα-R-Hpal	TTTGTTAACCTAATTAAATTACAAAAAGAAATAATCGAAGG			TV	5'PbGCα		
CT-GCα-F-SaclI	AAT <u>CCGCGG</u> CCGCTTAAAGATGATTATGG	0.4		TV	CT-PbGCα		
CT-GCα-R-Hpal	AA <u>GTTAAC</u> AAATTGAACTTGTGCTTGGAAAT			TV	CT-PbGCα		
3'GCα-F-AvrlI	AAT <u>CCT</u> AGGTAAATATTTCATGTTTACATAATTGC	0.5		TV	3'PbGCα		
3'GCα-R-KpnI	ATAGGTACCTTCATTGCTTAAATACGATAAGTCC			TV	3'PbGCα		
5'GCα-F	ATATTTGCCGATTTCATATGTATCC		0.8	GT	5'PbGCα		
5'GCα-R	TGTTATTTATTCAAGGGAAAGC	1.1		GT	5'PbGCα		
CT-GCα-F	ATCCAGTAGATGGAACAGAACG		1.4	0.7	GT	CT-PbGCα	
3'GCα-F	TGGAAAATTAAATGTCAGAAACC			GT	3'PbGCα		
3'GCα-R	TCATCTGTTTAAGGATGCAAGG		1.4	1.4	GT	3'PbGCα	
GCβ – PBANKA_113670 – PF3D7_1360500 – guanylyl cyclase beta (GCbeta)							
5'GCβ-F-SaclI	TTTCCGCGGTGTTGAAATAACATCATCAGTG	0.5		TV	5'PbGCβ		
5'GCβ-R-Hpal	TTTGTTAACCTAATTAAATTGAAAGTGGGCAAACAAAAGG			TV	5'PbGCβ		
3'GCβ-F-AvrlI	AAT <u>CCT</u> AGGTACAATGTCTGCAGAACGAG	0.5		TV	3'PbGCβ		
3'GCβ-R-KpnI	ATAGGTACCCGCAACAAAGAATTCTATAAAATG			TV	3'PbGCβ		
5'GCβ-F	CAAATTAAAGCGCGTATGC		1.0	GT	5'PbGCβ		
5'GCβ-R	GAATGCACATAGTTAACATCGC	1.1		GT	5'PbGCβ		
3'GCβ-F	TTACACTATGGTCGTGTTGG			GT	3'PbGCβ		
3'GCβ-R	AGCTTATGTTAAACGTATTACG		1.6	1.4	GT	3'PbGCβ	
MFS1 – PBANKA_123130 – PF3D7_0516500 – metabolite/drug transporter, putative							
5'MFS1-F-SaclI	TTTCCGCGGAAGGAATAAAGGAAAGGTTATGC	0.5		TV	5'PbMFS1		
5'MFS1-R-Hpal	TTTGTTAACCTAATTAAATTCTAAATTGACCCTAACAAACAG			TV	5'PbMFS1		
3'MFS1-F-AvrlI	AAT <u>CCT</u> AGGAATTTCGCTGACTATTCTTTGC	0.5		TV	3'PbMFS1		
3'MFS1-R-KpnI	ATAGGTACCTGATTGCTGTATGCAATTATTACG			TV	3'PbMFS1		
5'MFS1-F	CATATTCTATAAACATACACATAGTGG		0.9	GT	5'PbMFS1		
5'MFS1-R	TATTCGAACCATCATTGTTGG	1.2		GT	5'PbMFS1		
3'MFS1-F	TACAATAATGGCTCCCTATTGG/GTTGTAGATATGTTCAAGTAAACACC			GT	3'PbMFS1		
3'MFS1-R	TGAAGCATACGAAGAAAATTAAAGC	1.0	1.4	GT	3'PbMFS1		
MFS2 – PBANKA_081700 – PF3D7_0916000 – sugar transporter, putative							
5'MFS2-F-SaclI	TTTCCGCGGGGGACTAGAGACAAACAGTACC	0.5		TV	5'PbMFS2		
5'MFS2-R-Hpal	TTTGTTAACCTAATTAGAACATCAGCTCAATTACATGC			TV	5'PbMFS2		
3'MFS2-F-AvrlI	AAT <u>CCT</u> AGGTATACACACATTATGCTTATTTC	0.5		TV	3'PbMFS2		
3'MFS2-R-KpnI	ATAGGTACCGTATAGAGCCAAATAAGACG			TV	3'PbMFS2		
5'MFS2-F	GAGCACAACACATAACAGTGC		0.8	GT	5'PbMFS2		
5'MFS2-R	TCTTTTCAGTTTATCCATGTTAGC	0.9		GT	5'PbMFS2		
3'MFS2-F	TCGGAAAACATAATAGATCAGC			GT	3'PbMFS2		
3'MFS2-R	CATTCGTTAAATTACTACATTG		1.9	1.5	GT	3'PbMFS2	
MFS3 – PBANKA_082040 – PF3D7_0919500 – sugar transporter, putative							
5'MFS3-F-SaclI	TTTCCGCGGGATATTTGTTCTCTTCTATCTCC	0.5		TV	5'PbMFS3		
5'MFS3-R-Hpal	TTTGTTAACCTAATTAGTTGGTATATGTTGAAATTGTTTCG			TV	5'PbMFS3		
3'MFS3-F-AvrlI	AAT <u>CCT</u> AGGTCTCTTATTGTTATGTTAGACTTATC	0.5		TV	3'PbMFS3		
3'MFS3-R-KpnI	ATAGGTACCTGAATTAAGTGCAGTATG			TV	3'PbMFS3		
5'MFS3-F	CAGTCTTGAGTAATACTAGCC		0.9	GT	5'PbMFS3		
5'MFS3-R	AAACAAACTATAACATTGAGCATCC			GT	5'PbMFS3		
3'MFS3-F	TTGTATTACTATAACTAAACTAAATTGGC			GT	3'PbMFS3		
3'MFS3-R	GTTTAAAACATCTAATATTACTAAATCC		1.6	1.8	GT	3'PbMFS3	

Supplementary Table 3 | Primer sequences and PCR product sizes (continued...)

Primer Name	Primer Sequence (restriction sites underlined)	WT ^a	INT ^b	Use ^c	Target	Ref
MFS4 – PBANKA_060240 – PF3D7_1203400 – transporter, putative						
5'MFS4-F-SaclI	TTTCCGCGGACCATGCTGGTTTAATAAATTGG	0.5		TV	5'PbMFS4	
5'MFS4-R-Hpal	TTTGTTAACGTATGATTCTCGTATGTATGC			TV	5'PbMFS4	
3'MFS4-F-Avrl	AATCCTAGGGTATACAAATATAGCATCGATCCC	0.5		TV	3'PbMFS4	
3'MFS4-R-KpnI	ATAGGTACCTAGAATTTCATAAAATTGTATTG			TV	3'PbMFS4	
5'MFS4-F	ACTCTAAAATAACCTATACATACGCC	0.7	0.7	GT	5'PbMFS4	
5'MFS4-R	GGATATACACACACAATGTTATGTACC	0.7		GT	5'PbMFS4	
3'MFS4-F	AAATACGTTGGCTTGTACAGCTTCAGG	1.3		GT	3'PbMFS4	
3'MFS4-R	CAGAATTAGATCAGCTTCAGG		1.3	GT	3'PbMFS4	
MFS5 – PBANKA_101640 – PF3D7_1428200 – metabolite/drug transporter, putative						
5'MFS5-F-SaclI	TTTCCGGGGTCGTATTTAATGTTTCAAAATG	0.4		TV	5'PbMFS5	
5'MFS5-R-Hpal	TTTGTTAACCTTTCAATCCTGTATAACATCTTCG			TV	5'PbMFS5	
3'MFS5-F-Avrl	AATCCTAGGTCTGTTTAGCATATTATTGTCG	0.5		TV	3'PbMFS5	
3'MFS5-R-KpnI	ATAGGTACCATCTTAAATTGGTATACCGAAGC			TV	3'PbMFS5	
5'MFS5-F	ATAGTATATGTGCAATGATGAATTGG	0.7	0.8	GT	5'PbMFS5	
5'MFS5-R	TATCAATGATAAAATATTGACTATCCCC	0.7		GT	5'PbMFS5	
3'MFS5-F	GGAATGTTAAATGGAATACCAAAGC	1.4		GT	3'PbMFS5	
3'MFS5-R	ATTAAAACCAATTAGGACGATCG		1.3	GT	3'PbMFS5	
MFS6 – PBANKA_130470 – PF3D7_1440800 – major facilitator superfamily, putative						
5'MFS6-F-SaclI	TTTCCGCGGAATTAACATGCCATAAAATGTTCTC	0.6		TV	5'PbMFS6	
5'MFS6-R-Hpal	TTTGTTAACCTTTATTAAGAATGACTCTCCACC			TV	5'PbMFS6	
3'MFS6-F-Avrl	AATCCTAGGCATTATCGTGTATTTCTTC	0.5		TV	3'PbMFS6	
3'MFS6-R-KpnI	ATAGGTACCATATTTACGCCCTTTAGAAGC			TV	3'PbMFS6	
5'MFS6-F	CTTATTGCATATATAACTAAGAATGTGG	0.9	0.8	GT	5'PbMFS6	
5'MFS6-R	GCTGTAAAAGGACTCAAACAACG			GT	5'PbMFS6	
3'MFS6-F	CAAATACAAATTAGCCTTTGTTCC	1.5		GT	3'PbMFS6	
3'MFS6-R	GTAATATTTGATGCTGATTATAATACCC		1.3	GT	3'PbMFS6	
PAT – PBANKA_030390 – PF3D7_0206200 – metabolite/drug transporter, putative						
5'PAT-F-SaclI	TTTCCGCGGCCCTTCCCCGTTATGTAGC	0.4		TV	5'PbPAT	
5'PAT-R-Hpal	TTTGTTAACGAAATGAAACACTTAGGGTATGG			TV	5'PbPAT	
3'PAT-F-Avrl	AATCCTAGGGGACAATAATGGATACTTTAGATTG	0.6		TV	3'PbPAT	
3'PAT-R-KpnI	ATAGGTACCTGGGCATAGATTCTTGTAGC			TV	3'PbPAT	
5'PAT-F	GATTATTACAAGTATACTTACATTACACC	0.6	0.6	GT	5'PbPAT	
5'PAT-R	ATTTTCTCTCAATACATGGGACC			GT	5'PbPAT	
3'PAT-F	GGATTTACTGGTACAATCCATGG	1.4		GT	3'PbPAT	
3'PAT-R	TTAACCTGCGAAAAGAAAACCTCG		1.4	GT	3'PbPAT	
MFR1 – PBANKA_011250 – PF3D7_0614300 – organic anion transporter						
5'MFR1-F-SaclI	TTTCCGCGGTCTATTAAACAAATTGAAAGGGATGAC	0.6		TV	5'PbMFR1	
5'MFR1-R-Hpal	TTTGTTAACATTAAATAATAAAGTATATATTATGTTATTTGCTTC			TV	5'PbMFR1	
3'MFR1-F-Avrl	AATCCTAGGGCACATATTCTAGTATTTCATTATACGC	0.5		TV	3'PbMFR1	
3'MFR1-R-KpnI	ATAGGTACCGGGCCCATTTCTTGTATTTC			TV	3'PbMFR1	
5'MFR1-F	AAATACTAGGTTAATGAATTGTATATAACCC	1.3	0.9	GT	5'PbMFR1	
5'MFR1-R	AAAGGGATCATTTATATGCTAAGG			GT	5'PbMFR1	
3'MFR1-F	TTTTGGGATATACTGCTTTATCG	1.7		GT	3'PbMFR1	
3'MFR1-R	CTTAAATAGTGAACATTAATGTGGG		1.5	GT	3'PbMFR1	
MFR2 – PBANKA_020840 – PF3D7_0104700 – transporter, putative						
5'NPT1-F-SaclI	TTTCCGCGCGTGCATGCTTATAC	0.5		TV	5'PbNPT1	
5'NPT1-R-Hpal	TTTGTTAACAAAAATAATTTCGTTGATGTATGC			TV	5'PbNPT1	
3'NPT1-F-Avrl	AATCCTAGGGCACATATTCTAGTATTTCATTATACG	0.5		TV	3'PbNPT1	
3'NPT1-R-KpnI	ATAGGTACCAATATTTCGATTTGTCCCTGTTCATATC			TV	3'PbNPT1	
5'NPT1-F	TATATATGCACTAACTAGCTTCG	1.0	1.1	GT	5'PbNPT1	
5'NPT1-R	TTTATTCCTTTCGATATTCTCC			GT	5'PbNPT1	
3'NPT1-F	GGAAAATCAGGAGAGGATTACG	1.3		GT	3'PbNPT1	
3'NPT1-R	CGAAAATATTGTATACTACCAAGAAC		1.7	GT	3'PbNPT1	
MFR3 – PBANKA_041050 – PF3D7_0312500 – transporter, putative						
5'MFR3-F-SaclI	TTTCCGCGGCAATAAAATAGCAAATGGAAAAGAGG	0.5		TV	5'PbMFR3	
5'MFR3-R-Hpal	TTTGTTAACCTCTCTATAATCCCTTATAGCC			TV	5'PbMFR3	
3'MFR3-F-Avrl	AATCCTAGGGTATTTAAACAGTTCAGAAACATTCCC	0.5		TV	3'PbMFR3	
3'MFR3-R-KpnI	ATAGGTACCTATGTTCTAAACAGAATTGAGCC			TV	3'PbMFR3	
5'MFR3-F	ATAATTGTTAATATTGTAACATAACAAAAGG	0.7	0.9	GT	5'PbMFR3	
5'MFR3-R	TTGCACATTTCGATTAATTCCG			GT	5'PbMFR3	
3'MFR3-F	AGAATTCTTATTTAACATTAGTAAATTGC	1.2		GT	3'PbMFR3	
3'MFR3-R	TTTTCACTTCTAAACTACATATACG		1.3	GT	3'PbMFR3	

Supplementary Table 3 | Primer sequences and PCR product sizes (continued...)

Primer Name	Primer Sequence (restriction sites underlined)	WT ^a	INT ^b	Use ^c	Target	Ref
MFR4 – PBANKA_081570 – PF3D7_0914700 – transporter, putative						
5'MFR4-F-SaclI	TTTCCGCGGGATAAAGTTTATTTAAACATCATTGTGC	0.6		TV	5'PbMFR4	
5'MFR4-R-Hpal	TTTGTTAACCTAATTAAATGAAATTAAAATTTGGCTACTTTGG			TV	5'PbMFR4	
3'MFR4-F-AvrlI	AATCCTAGGGTAACACTCGAATTATGCACATCC	0.5		TV	3'PbMFR4	
3'MFR4-R-KpnI	ATAGGTACCAATTCTGAACATGTCTAAAAATAAAGG			TV	3'PbMFR4	
5'MFR4-F	GTATTGTTGATAATTGGGACATGC	1.3	1.1	GT	5'PbMFR4	
5'MFR4-R	AACTCGAAATGATTCTATTACATCG			GT	5'PbMFR4	
3'MFR4-F	ATAGGAAGTGAATTGCTACTGG	1.7		GT	3'PbMFR4	
3'MFR4-R	TGTGTTATGAAGATACACCTTC		1.5	GT	3'PbMFR4	
MFR5 – PBANKA_091830 – PF3D7_1129900 – transporter, putative						
5'MFR5-F-SaclI	TTTCCGCGGTGCAACAAAAGTATAACAACATCG	0.6		TV	5'PbMFR5	
5'MFR5-R-Hpal	TTTGTTAACCTAATTAAAGCTTAATTCGATATTATTATATGGTATTG			TV	5'PbMFR5	
3'MFR5-F-AvrlI	AATCCTAGGTATGCCGTGATTTGATTATGC	0.5		TV	3'PbMFR5	
3'MFR5-R-KpnI	ATAGGTACCCCATTTCTTATCATTTTCAACTCC			TV	3'PbMFR5	
5'MFR5-F	TTAAAATAGGGCAGAGATAATAAAGG	1.5	1.3	GT	5'PbMFR5	
5'MFR5-R	TGACCCAAATAAAAATTGTTGAACC			GT	5'PbMFR5	
3'MFR5-F	TTTCCCAGATCTAGATATTAGG	1.6		GT	3'PbMFR5	
3'MFR5-R	AATTCTTGATAACGTTGAAAAGC		1.4	GT	3'PbMFR5	
NPT1 – PBANKA_020830 – PF3D7_0104800 – novel putative transporter 1 (NPT1)						
5'MFR2-F-SaclI	TTTCCGCGGAATGAATATGTTTGCACCTTATTATATGC	0.5		TV	5'PbMFR2	
5'MFR2-R-Hpal	TTTGTTAACCTAATTAAATATTGCGAAAACCTTGGTATGG			TV	5'PbMFR2	
3'MFR2-F-AvrlI	AATCCTAGGTGTAGTCCAGATTATTCAAATTGC	0.5		TV	3'PbMFR2	
3'MFR2-R-KpnI	ATAGGTACCCATTCTAGGATCCTCCTTTGG			TV	3'PbMFR2	
5'MFR2-F	CACAAAGTTAAGGAATATGAATGC	0.6	0.7	GT	5'PbMFR2	
5'MFR2-R	TGTACAATTGATTCTTCATTCTCG			GT	5'PbMFR2	
3'MFR2-F	AAATTCAAGTGGAAATAAAATGATCC/ AGAGATGCAACCATCTTAATCC	1.4		GT	3'PbMFR2	
3'MFR2-R	CATTCTCTTCAGGAAAGTCC		1.3	GT	3'PbMFR2	
CDF – PBANKA_142220 – PF3D7_0715900 – zinc transporter, putative						
5'CDF-F-SaclI	TTTCCGCGGATTTACATAGGAATATAACAAAACAAGC	0.6		TV	5'PbCDF	
5'CDF-R-Hpal	TTTGTTAACCTAATTAAATCTTATTTAAACTCTTAGTACCG			TV	5'PbCDF	
3'CDF-F-AvrlI	AATCCTAGGTGCCCCAAAAGTAGCTAACGC	0.5		TV	3'PbCDF	
3'CDF-R-KpnI	ATAGGTACCCAAATGGTAGAGTGCAGAAAATCG			TV	3'PbCDF	
5'CDF-F	GCTGCCTATTCAAACATACTACC	1.1	0.9	GT	5'PbCDF	
5'CDF-R	AAACAAAGCTCCATAATTCTGC			GT	5'PbCDF	
3'CDF-F	TCCACCTAATGTTAAAGTAGGC	1.6		GT	3'PbCDF	
3'CDF-R	TATCAGCTCAAACAAATAATTATTGG		1.4	GT	3'PbCDF	
ZIP1 – PBANKA_010770 – PF3D7_0609100 – Zn²⁺ or Fe²⁺ permease						
5'ZIP1-F-SaclI	TTTCCGCGGATAGTTTGAAGGGAAACAATTAGCC	0.6		TV	5'PbZIP1	
5'ZIP1-R-Hpal	TTTGTTAACCTAATTAAAAGAAATCTAGGCCCTATATTGC			TV	5'PbZIP1	
3'ZIP1-F-AvrlI	AATCCTAGGGGCCATAATATAAGCAAAGAACG	0.5		TV	3'PbZIP1	
3'ZIP1-R-KpnI	ATAGGTACCCAAATAATTAGAAACCTTGTGGATTATGG			TV	3'PbZIP1	
5'ZIP1-F	GGTTTGTAGAAATGAATAACATCG	1.1	1.1	GT	5'PbZIP1	
5'ZIP1-R	TCTTCCAAAATAATCCAAAATATAAGG			GT	5'PbZIP1	
3'ZIP1-F	GGGATTGAAGGTAGATAAATAAAAAGG	1.7		GT	3'PbZIP1	
3'ZIP1-R	TGAAAAAAATTGCAAATACCTATTGG		1.4	GT	3'PbZIP1	
DMT1 – PBANKA_142210 – PF3D7_0715800 – drug/metabolite exporter, drug/metabolite transporter						
5'DMT1-F-SaclI	TTTCCGCGGTGTGTCCTGGTTCTACACG	0.5		TV	5'PbDMT1	
5'DMT1-R-Hpal	TTTGTTAACCTAATTAAATTATGGAAAGCAAATAACTATACC			TV	5'PbDMT1	
3'DMT1-F-AvrlI	AATCCTAGGCCTGGTGAATGTATCTATTGAGC	0.5		TV	3'PbDMT1	
3'DMT1-R-KpnI	ATAGGTACCCGCTAAAGAAAGAGCATGTAGG			TV	3'PbDMT1	
5'DMT1-F	TCACTTGCTTGTGTTATATTCC	1.0	0.7	GT	5'PbDMT1	
5'DMT1-R	ATACAAAAGCATAGAAAACAAAAGC			GT	5'PbDMT1	
3'DMT1-F	TTTTAGCTATATTGTTATCCTTATTGG	1.3		GT	3'PbDMT1	
3'DMT1-R	GGAATTTTATCAAATAAACATTCGG		1.4	GT	3'PbDMT1	

Supplementary Table 3 | Primer sequences and PCR product sizes (continued...)

Primer Name	Primer Sequence (restriction sites underlined)	WT ^a	INT ^b	Use ^c	Target	Ref
DMT2 – PBANKA_061460 – PF3D7_0716900 – drug metabolite transporter, putative						
5'DMT2-F-SaclI	TTTCGCGGGCGTAAATAATGTTCACATTCTACC	0.6		TV	5'PbDMT2	
5'DMT2-R-Hpal	TTGTTAACCTAATTAACTAAATATGAAATGTTATGCTGTTTTC			TV	5'PbDMT2	
CT-DMT2-F-SaclI	AATCCGGGGAAAAATATATGCATAAAATGGATGGAC	0.5		TV	CT-PbDMT2	
CT-DMT2-R-Hpal	AAAGTTAACCTACCTTGGCTTAATTAAAGATATCTG			TV	CT-PbDMT2	
3'DMT2-F-AvrlI	AATCCTAGGCAGATCACCAGTTAACCAATTAAAG	0.5		TV	3'PbDMT2	
3'DMT2-R-KpnI	ATAGGTACCCACATACATAATGTCAAGCAAACATAG			TV	3'PbDMT2	
5'DMT2-F	TACAACAATGTGGTATTATATATTATCATCC		0.9	GT	5'PbDMT2	
5'DMT2-R	AACAAACAATAATGTTGCTTCCACC	0.8		GT	5'PbDMT2	
CT-DMT2-F	as 3'DMT2-F	1.4	0.7	GT	CT-PbDMT2	
3'DMT2-F	CAAATACTTAAAGTCGAAATACATGG			GT	3'PbDMT2	
3'DMT2-R	GTATACTGAATATACATTAAAACATCACC	1.4	1.4	GT	3'PbDMT2	
GAP40 – PBANKA_11530 – PF3D7_0515700 – glideosome-associated protein 40, putative (GAP40)						
5'GAP40-F-SaclI	TTTCGCGGGTGCATAATTAAACAAATTAAATTATTAGC	0.5		TV	5'PbGAP40	
5'GAP40-R-Hpal	TTGTTAACCTTATTACTAAATTAAATAACAGATTGTC			TV	5'PbGAP40	
3'GAP40-F-AvrlI	AATCCTAGGGTGTATGTGCTCATTCATTGC	0.5		TV	3'PbGAP40	
3'GAP40-R-KpnI	ATAGGTACCCGCAAACAGAAATCTATAAGTCC			TV	3'PbGAP40	
5'GAP40-F	CAATCTCAAAGTACACACAATTCC		0.8	GT	5'PbGAP40	
5'GAP40-R	ACTCTGGCAAATTGACTTTCC	0.7		GT	5'PbGAP40	
3'GAP40-F	TTTATGATTGGTTAGGATATAATAGACC			GT	3'PbGAP40	
3'GAP40-R	TTATGGGAATTCTTTCTCATGC	1.3	1.4	GT	3'PbGAP40	
TPT3 – PBANKA_143400 – PF3D7_1218400 – triose or hexose phosphate/phosphate translocator, putative						
5'TPT3-F-SaclI	TTTCGCGGGATTGTTCATTTATATTCTTATCTTTAAACC	0.6		TV	5'PbTPT3	
5'TPT3-R-Hpal	TTGTTAACCTAATTAAATGTTAGTCACAAAAAAATTAAACAGATTGTC			TV	5'PbTPT3	
3'TPT3-F-AvrlI	AATCCTAGGTGTATGTGCTCATTCATTCC	0.5		TV	3'PbTPT3	
3'TPT3-R-KpnI	ATAGGTACCGTCCCTCACGTGTATGTGC			TV	3'PbTPT3	
5'TPT3-F	AAAGGAAGAACAGTAGCAAATGG		1.0	GT	5'PbTPT3	
5'TPT3-R	TTGTTGATCATCTTCATTGTTATTCATTGG	0.8		GT	5'PbTPT3	
3'TPT3-F	GATTTCCATTATTGTGGCTATATGC			GT	3'PbTPT3	
3'TPT3-R	TGGATCAAATACCTACTGAATCG	1.3	1.8	GT	3'PbTPT3	
NT2 – PBANKA_070620 – PF3D7_0824400 – nucleoside transporter 2 (NT2)						
5'NT2-F-SaclI	TTTCGCGGGAGCATGTATGTTAGTTAAAGCAG	0.5		TV	5'PbNT2	
5'NT2-R-Hpal	TTGTTAACCTAATTAAACGAAAAAAAAATATATATTAAAGGCTT			TV	5'PbNT2	
3'NT2-F-AvrlI	AATCCTAGGATACATTTTAATGCCTAAATTGAACG	0.4		TV	3'PbNT2	
3'NT2-R-KpnI	ATAGGTACCGCATGTGGTAATAAAATTATGATAGG			TV	3'PbNT2	
5'NT2-F	TTATTTATTGGGATAAGAGAACTCC	0.7	0.8	GT	5'PbNT2	
5'NT2-R	CATTGGAAATATGCTGTGC			GT	5'PbNT2	
3'NT2-F	ATAAGTGGAAACTCAACTTCAGC			GT	3'PbNT2	
3'NT2-R	TTGGAAAATTGTAAGAAATTGTTATCC	1.6	1.6	GT	3'PbNT2	
NT4 – PBANKA_020990 – PF3D7_0103200 – nucleoside transporter 4 (NT4)						
5'NT4-F-SaclI	TTTCGCGGAAATTATTATTGATCCATCATGTTGG	0.5		TV	5'PbNT4	
5'NT4-R-Hpal	TTGTTAACCTAATTAAATCTGGGTATGTATATTTCCTTAGC			TV	5'PbNT4	
3'NT4-F-AvrlI	AATCCTAGGTGTGGCCAATTGTTATCTTCC	0.5		TV	3'PbNT4	
3'NT4-R-KpnI	ATAGGTACCTTTAACACTTATCATCGCTGG			TV	3'PbNT4	
5'NT4-F	GATATCGCAATTGGTATTATATGG	1.4	1.1	GT	5'PbNT4	
5'NT4-R	CAAATAATGCGAAACTAAATTCC			GT	5'PbNT4	
3'NT4-F	GTAAACCAACTAAATGATGTAGAAAAGG			GT	3'PbNT4	
3'NT4-R	TTCTTAAAGGTAAACAAAATTACGC	1.6	1.5	GT	3'PbNT4	
MATE – PBANKA_030970 – PF3D7_0212800 – multidrug efflux pump, putative						
5'MATE-F-SaclI	TTTCGCGGAAAAATCCAAATTATCATAAAAGAAAGC	0.5		TV	5'PbMATE	
5'MATE-R-Hpal	TTGTTAACCTAATTAACTACTGGTATTCTCCAAAGATTTC			TV	5'PbMATE	
3'MATE-F-AvrlI	AATCCTAGGGACTCAAATTCCCACCTTTTATACG	0.5		TV	3'PbMATE	
3'MATE-R-KpnI	ATAGGTACCTTAACTAAATATATTATACTTCTCATGGGG			TV	3'PbMATE	
5'MATE-F	GAACCTAACCTAAAGCAAATAGC		1.2	GT	5'PbMATE	
5'MATE-R	CCCTCAACTACAGAATTAAATAGC	1.5		GT	5'PbMATE	
3'MATE-F	AATTAAACATCTGTTGCAAGTATTATCC			GT	3'PbMATE	
3'MATE-R	GAACCTTTGTTACTAGCAACTGG	1.7	1.8	GT	3'PbMATE	

^a Sizes of the PCR products of forward and reverse primers on WT gDNA; carboxy-terminal *mtp::tag* integration-specific primers combined with their respective reverse 3' gene-specific primers.

^b Sizes of the respective integration-specific PCR products; forward 5' and carboxy-terminal gene-specific primers combined with 5'HSP70rev (*mtp*⁻ lines) or mCherryRev (*mtp::tag* lines) and reverse 3' gene-specific primers combined with 5'DHFRrev.

^c TV, primers used for construction of Transfection Vectors; GT, primers used for GenoTyping; SQ, primers used for sequencing the targeting sequences.

^d For WT-specific diagnostic PCR the 5'CTR2-F and 3'CTR2-R were combined to amplify the complete locus.

SUPPLEMENTARY REFERENCES

1. Ren, Q., Chen, K. & Paulsen, I. T. TransportDB: a comprehensive database resource for cytoplasmic membrane transport systems and outer membrane channels. *Nucleic Acids Res.* **35**, D274–9 (2007).
2. Frénal, K. *et al.* Functional dissection of the apicomplexan glideosome molecular architecture. *Cell Host Microbe* **8**, 343–357 (2010).
3. Fonager, J. *et al.* Development of the *piggyBac* transposable system for *Plasmodium berghei* and its application for random mutagenesis in malaria parasites. *BMC Genomics* **12**, 155 (2011).
4. Choveaux, D. L., Przyborski, J. M. & Goldring, J. D. A *Plasmodium falciparum* copper-binding membrane protein with copper transport motifs. *Malar. J.* **11**, 397 (2012).
5. Tran, P. N. *et al.* A female gametocyte-specific ABC transporter plays a role in lipid metabolism in the malaria parasite. *Nat. Commun.* **5**, 4773 (2014).
6. Zalis, M. G., Wilson, C. M., Zhang, Y. & Wirth, D. F. Characterization of the *pfmdr2* gene for *Plasmodium falciparum*. *Mol. Biochem. Parasitol.* **62**, 83–92 (1993).
7. Trottein, F. & Cowman, A. F. Molecular cloning and sequence of two novel P-type adenosinetriphosphatases from *Plasmodium falciparum*. *Eur. J. Biochem.* **227**, 214–225 (1995).
8. Fonager, J. *et al.* Reduced CD36-dependent tissue sequestration of *Plasmodium*-infected erythrocytes is detrimental to malaria parasite growth *in vivo*. *J. Exp. Med.* **209**, 93–107 (2012).
9. Carucci, D. J. *et al.* Guanylyl cyclase activity associated with putative bifunctional integral membrane proteins in *Plasmodium falciparum*. *J. Biol. Chem.* **275**, 22147–22156 (2000).
10. Hirai, M., Arai, M., Kawai, S. & Matsuoka, H. *PbGC β* is essential for *Plasmodium* ookinete motility to invade midgut cell and for successful completion of parasite life cycle in mosquitoes. *J. Biochem.* **140**, 747–757 (2006).
11. Moon, R. W. *et al.* A cyclic GMP signalling module that regulates gliding motility in a malaria parasite. *PLoS Pathog.* **5**, e1000599 (2009).
12. Augagneur, Y. *et al.* Identification and functional analysis of the primary pantothenate transporter, *PfPAT*, of the human malaria parasite *Plasmodium falciparum*. *J. Biol. Chem.* **288**, 20558–20567 (2013).
13. Hart, R. J., Lawres, L., Fritzen, E., Ben Mamoun, C. & Aly, A. S. I. *Plasmodium yoelii* vitamin B5 pantothenate transporter candidate is essential for parasite transmission to the mosquito. *Sci. Rep.* **4**, 5665 (2014).
14. Boisson, B. *et al.* The novel putative transporter NPT1 plays a critical role in early stages of *Plasmodium berghei* sexual development. *Mol. Microbiol.* **81**, 1343–1357 (2011).
15. Downie, M. J. *et al.* *PfNT2*, a permease of the equilibrative nucleoside transporter family in the endoplasmic reticulum of *Plasmodium falciparum*. *J. Biol. Chem.* **285**, 20827–20833 (2010).
16. Frame, I. J., Merino, E. F., Schramm, V. L., Cassera, M. B. & Akabas, M. H. Malaria parasite type 4 equilibrative nucleoside transporters (ENT4) are purine transporters with distinct substrate specificity. *Biochem. J.* **446**, 179–190 (2012).
17. Haussig, J. M., Matuschewski, K. & Kooij, T. W. A. Experimental genetics of *Plasmodium berghei* NFU in the apicoplast iron-sulfur cluster biogenesis pathway. *PLoS ONE* **8**, e67269 (2013).
18. Kenthirapalan, S., Waters, A. P., Matuschewski, K. & Kooij, T. W. A. Flow cytometry-assisted rapid isolation of recombinant *Plasmodium berghei* parasites exemplified by functional analysis of aquaglyceroporin. *Int. J. Parasitol.* **42**, 1185–1192 (2012).